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The Solubilities of β-Lactoglobulins A, B, and AB

J. M. TREECE,[1] R. S. SHEINSON, AND T. L. McMEEKIN

From the Eastern Regional Research Laboratory,[2] Philadelphia, Pennsylvania

The solubilities of β-lactoglobulins A and B in dilute sodium chloride solutions were found to vary when the ratio of total protein to the volume of solvent was varied, particularly when the amount of dissolved protein almost equalled the total protein present, indicating heterogeneity. However, with an excess of protein, characteristic solubilities for β-lactoglobulins A and B were obtained.

When synthetic mixtures of β-lactoglobulins A and B, varying widely in composition, were tested for homogeneity by the solubility method, they were found not to vary to any greater extent when the ratio of protein to solvent was varied than did the electrophoretically homogeneous components. Such mixtures had numerical solubility values which were related to the amount of each component present.

The solubilities of samples of β-lactoglobulin A, from individual cow's milk, were essentially the same in water and dilute sodium chloride solutions, being about one fifth of that of β-lactoglobulin B in corresponding solvents. If, however, the solubility in sodium chloride is divided by the solubility in water, the logarithm of the ratio is the same for any given salt concentration for both type A and B, or mixtures of the two, indicating that the number and kind of dipoles in the two types of β-lactoglobulin could be the same.

The isoionic point of β-lactoglobulin A was found to be 5.23, β-lactoglobulin B, 5.30, and pooled β-lactoglobulin, 5.28 when determined by the pH of their deionized solutions.

INTRODUCTION

The solubility of β-lactoglobulin has been used as a method for determination of its purity and for characterization since its isolation was described by Palmer (1). Early solubility studies in dilute salt solutions indicated that it was essentially homogeneous (2). However, it was later shown, by means of solubility in dilute salt solutions (3) and also by electrophoresis (4), to be a mixture of proteins. Heredity was shown to be a source of heterogeneity of β-lactoglobulin by Aschaffenburg and Drewry (5), who demonstrated by paper electrophoresis that β-lactoglobulin from individual cow's milk existed in three forms which they designated A, B, and AB based on mobilities. These β-lactoglobulins of different genetic origin have been shown to differ in content of glycine, aspartic acid, alanine, and valine (6, 7).

The present study was made to determine the homogeneity of β-lactoglobulins A and B prepared from typed individual cow's milk by the solubility method in dilute salt solutions and to compare the solubility behavior of these closely related proteins. Previous results have been reported on the heterogeneity of the genetic variants of β-lactoglobulin by the solubility method in concentrated salt solutions by a slightly different procedure (8, 9). The results herein reported confirm and extend these previous findings.

[1] Postdoctoral Resident Research Associate, Present address: School of Veterinary Medicine. University of Pennsylvania, New Bolton Center, Kennett Square, Pennsylvania.

[2] Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

MATERIALS AND METHODS

Electrophoretic typing of milk and β-lactoglobulin. The fat and casein were removed from small samples of milk by acidification to pH 4.7 and centrifugation. After the removal of lactose from the whey by dialysis, the solution was lyophilized. The electrophoretic pattern of the whey protein was determined by the horizontal starch gel method (10) with borate buffer, pH 8.6. The electrophoretic composition was also determined on the crystallized β-lactoglobulin by the starch gel method as well as by the cyanogum gel method of Raymond (11) as modified by Peterson (12). A 7% cyanogum gel containing tris-borate buffer, pH 9.2, was used.

Measurement of pH. A Beckman model G[3] pH meter with glass electrodes was used. It was standardized at pH 4.0 and 7.0 with a standard buffer at a temperature of 25°. Some of the pH measurements were also checked with the Radiometer TTT1a[3] autotitrator. The electrodes were washed until a reading of pH 5.8 was given for distilled water before making measurements on protein solutions.

Preparation of β-lactoglobulin. Milk obtained from individual Guernsey and Holstein cows was used. Milk from a single milking or the combined milk of two milking periods, amounting to 1.3–3.0 gallons, was used to prepare β-lactoglobulin. Variations of the method of Palmer (1) were employed with ammonium sulfate instead of sodium sulfate.

A somewhat shorter method was devised which is particularly advantageous when transportation of the milk is involved. The cream and casein as well as some of the whey proteins were precipitated by making milk to 1.7 *M* $(NH_4)_2SO_4$ by adding solid $(NH_4)_2SO_4$. After the removal of this precipitate by filtration through a porous filter paper, β-lactoglobulin and other whey proteins were precipitated from the filtrate by making to 3.3 *M* $(NH_4)_2SO_4$. For 7 liters of milk, 1694 gm of $(NH_4)_2SO_4$ was required to make to 1.7 *M* $(NH_4)_2SO_4$; and for the resulting filtrate of 6 liters, 1773 gm of $(NH_4)_2SO_4$ was used to make to 3.3 *M* $(NH_4)_2SO_4$. The insoluble protein was then removed by filtering through a fluted paper. The well-drained precipitate and filter paper were macerated with 300 cc of water, and the filter paper was removed on a Buchner funnel. The filter paper was extracted twice using 100 cc of water for each extraction. To the combined filtrates, amounting to about 750 cc and containing approximately 1.5–1.7 *M* $(NH_4)_2SO_4$, normal sulfuric acid was added to pH 4.0 and the precipitate was removed by centrifugation. The resulting precipitate was suspended in water and dissolved by adding normal ammonium hydroxide to pH 7 and in a volume of 350 cc. The solution was again made to pH 4 by adding sulfuric acid. The precipitate was removed by centrifugation and was largely α-lactalbumin, which could be crystallized by the method of Gordon and Ziegler (13). The combined filtrates obtained at pH 4 were dialyzed until the pH approached 5.0. An amorphous precipitate formed on dialysis was removed by centrifugation. β-Lactoglobulin A or AB crystallized in good yield when the pH was made to 5.2. β-Lactoglobulin B, however, because of its greater solubility and smaller concentration (14), did not crystallize without concentrating the solution, which was done by suspending the dialyzing membranes in air and evaporating with a fan. On further dialysis after concentrating, crystalline β-lactoglobulin B was obtained. Each of the β-lactoglobulin samples was recrystallized four times by dissolving in 0.1 *N* sodium chloride and dialyzing. They were stored at 4° under water and toluene.

Electrophoretic composition. The electrophoretic patterns of the purified β-lactoglobulins were repeatedly determined by both starch gel and cyanogum gel electrophoresis. It was found that the A and B preparations were free from each other, as shown in Fig. 1; however, a small component moving faster than the main component was obtained in some of the β-lactoglobulin A preparations. Several unsuccessful attempts were made to separate the fast moving A component.

Amino acid composition. The recrystallized residues after the solubility determination were used to analyze a number of the β-lactoglobulin samples for their amino acid content.[4] The results obtained for the β-lactoglobulin A and B were in good agreement with the published values (6, 7). The amino acid composition of one sample of β-lactoglobulin AB from an individual cow's milk was also determined.

Solubility determination. The protein was washed with solvent by placing a rapidly weighed sample of wet crystals in about 10 cc of the solvent in a 50-ml centrifuge tube containing a small magnetic bar. After stirring for a few minutes, the solvent was removed by centrifugation and was discarded. A fresh volume of solvent, usually 5 or 10 cc, was placed on the protein. The tube was then closed with a rubber cap, coated with a

[3] Mention of commercial names does not imply indorsement by the U. S. Department of Agriculture.

[4] We wish to thank Mr. N. J. Hipp of this Laboratory for making these results available to us.

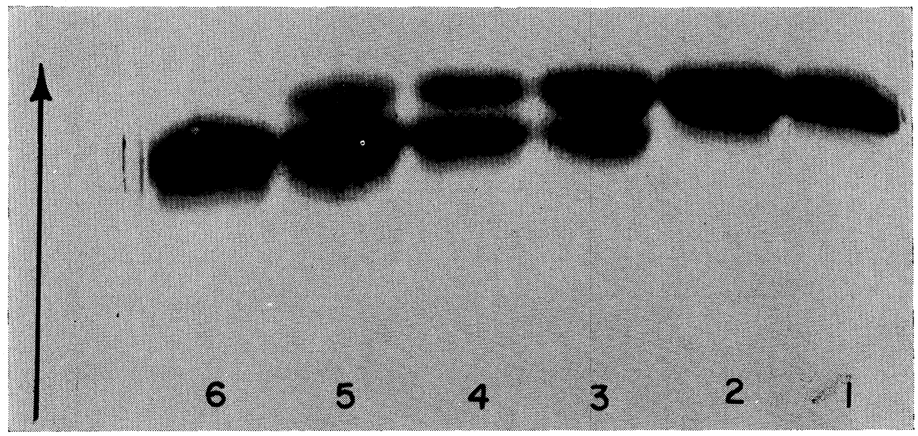

FIG. 1. Electrophoretic patterns of β-lactoglobulin. (1) Type A; (2) Type A; (3) 75% A–25% B; (4) 50% A–50% B (5) 25% A–75% B; (6) Type B.

thin film of silicone stopcock grease, and placed in a 25° ± 0.1 water bath. The suspension of β-lactoglobulin crystals was effectively stirred by means of a magnetic stirrer. Usually 24 hours was sufficient time for the solubility to reach equilibrium. Most of the β-lactoglobulin solutions were near the isoelectric point of pH 5.20. The pH of the solutions in salt varied from 5.15 for 0.025 M NaCl to 5.20 for 0.00625 M NaCl; however, in the solubilities in distilled water, the pH of the solution drifted to the alkaline side of the isoelectric point in the range of 5.30–5.48. After the period of equilibration (usually 18 hours followed by a second period of 7 hours), clear supernatants were obtained by centrifugation in an angle head at 4600 rpm for 5 minutes. The β-lactoglobulin concentration was determined by absorption at 278 mμ, using an extinction coefficient of $E^{1\%}_{1\mathrm{cm}} = 9.7$ based on the value of 15.6% for the nitrogen content of β-lactoglobulin. The values obtained for the solubilities of the β-lactoglobulins by absorption at 278 mμ were in good agreement with the results obtained by total nitrogen determinations. Most of the solubilities were determined by absorption at 278 mμ. For the measurement of absorption at 278 mμ, the sample was diluted with 0.005 N NaOH, giving a pH of 7.5–8.0.

Errors in solubility due to bacterial contamination were minimized by heating the glassware at 110° before using and by keeping the time of the solubility equilibration to a minimum. Germicides were not used since they would affect the solubility as well as interfere with the determination of the protein at 280 mμ. Bacterial contamination leads to higher solubilities due to ammonia production and can be detected by the odor as well as by an increase in the pH of the solutions with time.

RESULTS

A total of 19 preparations of β-lactoglobulins were studied: 7 type A, 9 type B, 2 type AB, and 1 from pooled milk. Figure 1 illustrates the results of a typical electrophoretic analysis in cyanogum gel; the procedure described by Peterson (12) was used.

SOLUBILITIES

For determining the effect of the ratio of the protein to the solvent on solubility, varying amounts of protein were added to a constant volume of solvent. Preliminary solubility results indicated that the pH of the dissolved protein in 0.00625 M sodium chloride was 5.20 after equilibrating for 24 hours, but the pH of equilibrated solutions in distilled water rose to 5.30–5.48. Consequently, the solubility in 0.00625 M sodium chloride was generally used in determining the effect of varying the amount of protein on solubility. The total protein was varied up to approximately 10-fold for a constant solvent volume. The results obtained are illustrated in Fig. 2. When the solubility of the protein is near that of the total protein present, it varies considerably with the total amount of protein. However, if there is a twofold or more excess of total protein, little change is found in solubility on adding more protein. The purity of the A and B prepara-

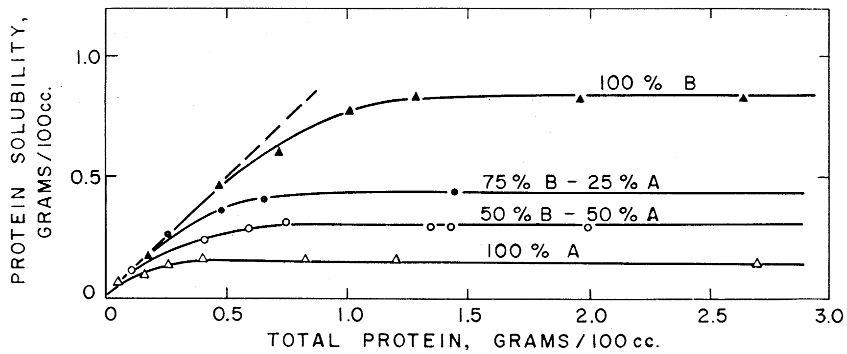

FIG. 2. Solubilities of β-lactoglobulins A and B and mixtures in 0.00625 M NaCl as a function of the total amount of protein present.

tions were similar by the solubility test although the B preparation was much more soluble than the A.

Since the β-lactoglobulin preparations appeared to be impure by the solubility test, it was of interest to determine the effect of artificial mixtures of A and B on solubility. Mixtures of A and B β-lactoglobulin, dissolved in sodium chloride, were made in definite proportions and were recrystallized four times by dialysis. The supernatants from the crystals were recovered by lyophilization in each case. Electrophoresis patterns (Fig. 1) on the 4-times crystallized mixtures of β-lactoglobulins, as well as on each of the supernatants, indicated that the components were present in the proportions in which they were mixed and that essentially no fractionation was attained by recrystallization. The finding that the supernatants from the recrystallization of synthetic mixtures of the A and B forms do not contain largely the B form is contrary to the results of Ogston and Tombs (15). This difference in findings is probably a result of the method of recrystallizing. The solubilities of the recrystallized mixtures, as well as the individual β-lactoglobulins A and B, were determined in 0.00625 M sodium chloride; in each case varying amounts of protein for a constant volume of 10 cc were used. The results in Fig. 2 show that the solubility is essentially independent of the amount of protein present when the amount present is 2–3 times that required to saturate the solution and that equilibrium is reached in 24 hours. It was also shown by electrophoresis that individual

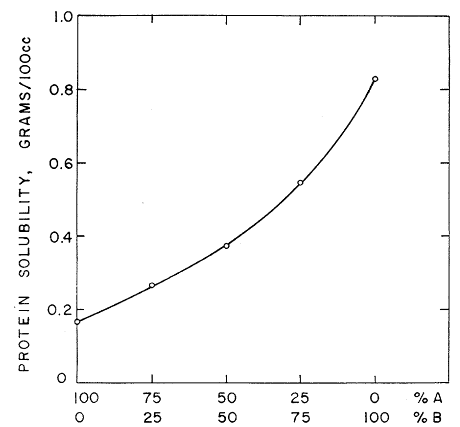

FIG. 3. Solubilities of β-lactoglobulins A and B and artificial mixtures in 0.00625 M NaCl when several-fold excess of undissolved protein is present.

crystals contained both A and B β-lactoglobulins and that the preparations were not mixtures of A and B crystals.

These results with different ratios of protein indicated the formation of a mixed crystal between the A and B forms of β-lactoglobulin. The solubilities were repeated using the same ratio of protein to solvent in each case, namely, 2.5 gm/100 cc. The results are given in Fig. 3 and are in essential agreement with the previous results (Fig. 2), showing that the solubility of these mixtures are related to the ratio of A and B types present. These results on solubility of mixtures of the two types of β-lactoglobulin crystallized together could be essentially duplicated by doing the solubility of mixtures of the two β-lactoglobulin A (Fig. 9). Palmer's value for the solubility of β-lactoglobulin in water of 1.23 gm per liter is considerably higher than can be explained on any basis.

The electrophoretic pattern obtained in borate buffer, pH 8.6, for Palmer's sample, as shown in Fig. 10, indicates that Palmer's sample of β-lactoglobulin is composed largely of type A, a small amount of B, and a component moving faster than A, which frequently occurs in samples of β-lactoglobulin A from individual cows. Even though the sample of β-lactoglobulin prepared by Palmer gave a complex electrophoretic pattern, its low solubility was consistent with the electrophoretic pattern and with Palmer's values for its solubility in salt.

Composition and Properties of β-Lactoglobulin AB

Aschaffenburg and Drewry (14) reported that the amount of β-lactoglobulin is considerably greater in type A milk than in type B from individual cows; consequently, it might be expected that AB-type cows would secrete the two forms in a ratio similar to the amounts present in the milk of the parent A and B types. The solubility data in Table I and Fig. 3 show that the solubility of β-lactoglobulin AB is nearer to that of A than B, which is consistent with the idea that β-lactoglobulin AB from individual cows has a larger amount of A than B. The determination of the relative quantities of A and B present in an electrophoretic pattern of β-lactoglobulin AB by means of a densitometer showed that the β-lactoglobulin A concentration was about twice as great as the B. It was found by amino acid analyses on one sample of β-lactoglobulin AB that the aspartic acid and valine content was the same as that of β-lactoglobulin A while the alanine content was the same as that of β-lactoglobulin B and the glycine content halfway between A and B. These results for the critical amino acids of the genetic variants of β-lactoglobulin indicate that amino acid analysis is not sufficiently accurate to determine the proportions of A and B in β-lactoglobulin AB.

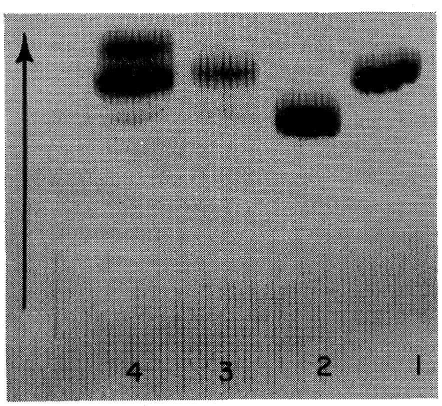

Fig. 10. Electrophoretic patterns of β-lactoglobulins A (1); B (2); and the sample of β-lactoglobulin prepared by Palmer; (3) most soluble crystals; (4) least soluble crystals, used in solubility determination.

DISCUSSION

The results obtained by the solubility test in dilute sodium chloride for homogeneity of the genetically different β-lactoglobulins are similar to the previous results on β-lactoglobulin prepared from mixed milk (1–3) though the numerical solubility values of the two forms are significantly different. In all cases reported, relatively constant solubilities could be obtained when a 2–3-fold excess of protein is used. The finding that electrophoretically homogeneous β-lactoglobulins were essentially as heterogeneous by the solubility method as natural or artificial mixtures of the two genetical forms indicates the solubility is not a good test for heterogeneity. The fact that β-lactoglobulins A and B form crystalline complexes in all proportions is consistent with the finding by Green *et al.* (22) that these two forms have the same unit cell dimensions. They reported that β-lactoglobulin B crystals change in crystalline form on standing to a form with a lower solubility. We have observed a change in size of the crystals of β-lactoglobulin B on standing, with a reduction in the rate of solution, though the equilibrium solubility was the same as the smaller crystals and was independent of the age of the crystals.

Our finding that the isoionic point of β-lactoglobulin A is 5.23 and B is 5.30 is in qualitative agreement with the corresponding values of 5.35 and 5.45 obtained by Tanford and Nozaki (16). This difference is small but appears to be significant since it persists in the presence of potassium chloride. The difference is possibly due to incomplete deionization or to the effect of protein concentration on its pH. In this connection, it is worthy of noting that in the determination of the solubility of the undeionized β-lactoglobulins in water, the pH of the solution was invariably between 5.30 and 5.48 even when the pH of the distilled water was made to pH 5.20 before determining the solubility.

In their comprehensive theoretical calculations describing the interaction of an electrolyte with dipolar ions, Scatchard and Kirkwood (23) have concluded that the negative logarithm of the activity coefficient —which for sparingly soluble ampholytes is equal to the logarithm of the quotient S_1/S_0, where S_1 is the solubility at a given ionic strength and S_0 the solubility at zero ionic strength—is directly proportional to the ionic strength and inversely proportional to the dielectric constant of the solution. The β-lactoglobulin solubility results represented in this manner (Fig. 6) follow a single curve for all genetic forms of β-lactoglobulin. This interpretation of the solubility data indicates that β-lactoglobulins A and B have the same number of dipoles; however, this evidence does not exclude other interpretations. The calculations of Cohn and Ferry (24) for a spherical molecule indicate that the negative logarithm of the activity coefficient increases with the square of the number of dipoles for a given salt concentration. Consequently, a difference in the number of dipoles in the A and B forms of β-lactoglobulin would be expected to produce a large effect in the solubility as expressed as the logarithm of S_1/S_0.

In the solubility determination, the pH of the solutions in salt have varied from pH 5.20 to 5.15, as shown in Table II; and in the absence of salt, the pH has risen as much as 0.25 unit above pH 5.20. It is thought that this variation in pH has not significantly influenced this interpretation of the solubility results on the β-lactoglobulins since Grönwall (3) found that a change of 0.2 pH unit had only a slight effect on the solubility of β-lactoglobulin in dilute salt and that the effect on solubility decreased with decreased salt concentration.

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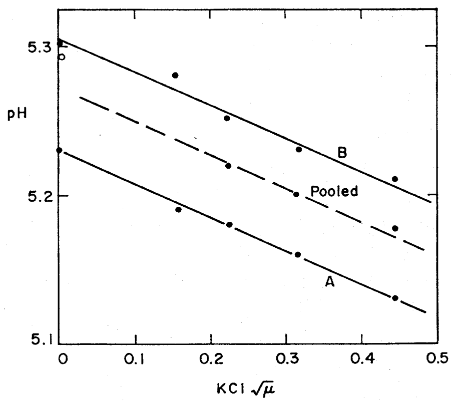

FIG. 7. The effect of KCl on the pH of deionized solutions of β-lactoglobulin's protein concentration of about 0.3%.

tive agreement with Tanford and Nozaki (16). The average value for the isoionic point of five preparations of A was found to be 5.23 ± 0.02, 5.30 for five preparations of B, and 5.28 for one preparation of pooled β-lactoglobulin. These values, as well as the effect of potassium chloride on their pH values, are recorded in Fig. 7. The protein concentrations varied somewhat but averaged about 0.3 %. The pH of the deionized solutions were measured immediately after they passed through the column since they were supersaturated and crystallized on standing. The effect of potassium chloride on the pH value of isoionic β-lactoglobulin solutions is small and amounts to only 0.1 pH for a 0.2 *M* solution of potassium chloride. The values for pooled β-lactoglobulin in potassium chloride are within the experimental error of ±0.02 pH of 5.20, in agreement with Cannan *et al.* (17). Tanford and Nozaki (16) used a 0.25 % solution of β-lactoglobulin for passing down the column; however, the concentration of the protein after passing down the column could have been smaller due to dilution and retardation. Adair and Adair (19) state that the value obtained for the isoionic point, as determined by the pH of the protein solution in water, is accurate only in high protein concentrations. According to Cannan (20), the concentration of the protein should not be less than 1 % when the pH of its solution in water is considered to be the isoionic point.

It was not possible to obtain deionized β-lactoglobulin solutions of a concentration of as great as 1 % because of their insolubility, though the tendency to form supersaturated solutions is sufficiently great to make possible pH measurements on solutions of much greater concentration than might be expected from equilibrium solubilities. It is of interest to note that the β-lactoglobulin solutions with a pH of 5.20 obtained from a mixed-bed resin column crystallized much quicker than the solutions with a slightly higher pH obtained by slower rates of flow. This observation is consistent with the finding of Grönwall (3) that the minimum solubility of β-lactoglobulin is obtained when small amounts of acid are present.

Since the concentration of β-lactoglobulin obtained in deionized solutions tends to be low, it was of interest to determine the effect of concentration of its pH. The variation of pH with concentration, as recorded in Fig. 8, illustrates the uncertainty involved in using the pH of a dilute protein solution as its isoionic point. Similar results were obtained with dilute solutions of β-lactoglobulin in 0.1 *M* sodium chloride solutions. Therefore, the concentration of the protein must also be stated when considering the effect of ionic strength on the pH of its solutions.

Crystalline preparations of each of the β-lactoglobulins were prepared from solutions that had been passed through mixed-bed deionizing columns by evaporating the solutions contained in cellophane tubing at room temperature and allowing them to stand at 4°. The solubilities of the deionized preparations were then determined in water and dilute sodium chloride solutions, and

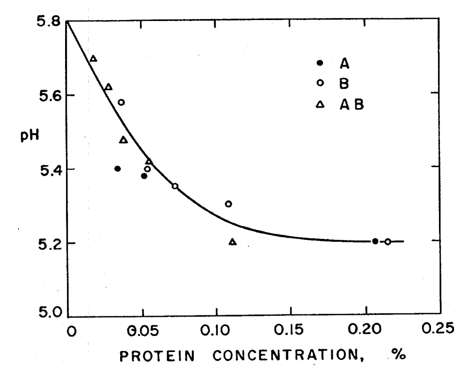

FIG. 8. Effect of protein concentration on the pH of deionized β-lactoglobulin solutions in water.

were found to be essentialy the same as similar preparations prepared by dialysis. These results indicate that β-lactoglobulin crystals obtained by exhaustive dialysis do not contain significant amounts of bound ions. The fact that the extrapolated values for the logarithm of the solubility of the β-lactoglobulins in water agree with the experimentally determined values when the solubility in salt is plotted on an ionic strength basis (Fig. 5) is consistent with the idea that these β-lactoglobulin preparations did not contain significant amounts of salt.

Solubility of β-Lactoglobulin Prepared by A. H. Palmer

The values reported by Palmer for the solubility of the original preparation of β-lactoglobulin in sodium chloride are much lower than have been reported by subsequent investigators (3, 21). Since it was found in this investigation that β-lactoglobulin A is much less soluble than the AB or B type, it appeared likely that the milk used by Palmer for preparing β-lactoglobulin came from predominantly type A cows. A sample of the β-lactoglobulin prepared by Palmer (1) many years ago was obtained from Dr. Robert C. Warner of the New York University Medical School. The wet crystals had been kept in a closed bottle with toluene in a refrigerator. The sample was almost entirely soluble in acetic acid at pH 4, and, on dialysis, it partially crystallized at pH 4.7 and completely at the isoelectric of pH 5.2, resembling β-lactoglobulin A in its low solubility in solutions acid to the isoelectric point. It was crystallized a second time by dissolving in dilute sodium chloride and removing the salt by dialysis. The solubility of this twice-crystallized β-lactoglobulin was determined by the previously described method. The results are given in Table II.

TABLE II

Solubility of β-Lactoglobulin Sample Prepared by A. H. Palmer

Conc. NaCl (M)	Solubility[a] (gm/1000 ml)	Log solubility	pH of solution
0.00	0.62	$\bar{1}.792$	5.30
0.0063	1.75	0.243	5.20
0.0125	3.59	0.555	5.17
0.025	9.75	0.989	5.15

[a] Temp.: 25°.

The values for the solubility of Palmer's sample of β-lactoglobulin are in fair agreement with the values reported by Palmer (1) for its solubility in salt and in good agreement with the values for the solubility of

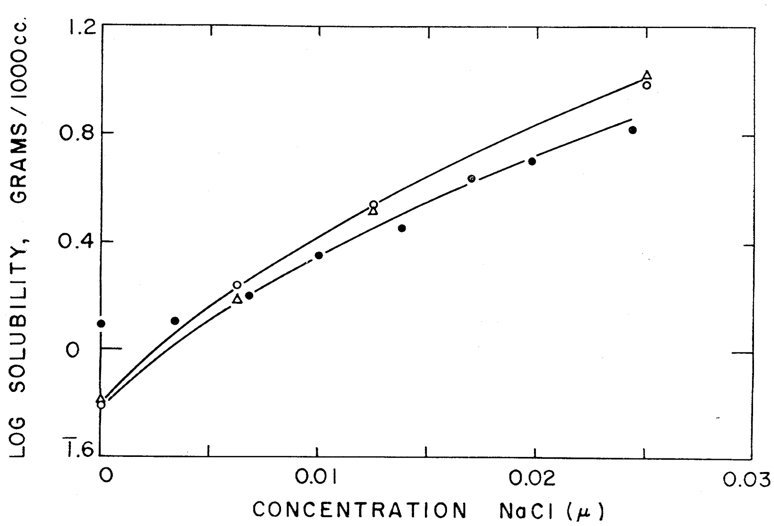

Fig. 9. Comparison of the published solubility data (●) of Palmer (1) with our results (○) on a recrystallized sample of β-lactoglobulin prepared by Palmer and the solubility (△) of β-lactoglobulin A.

TABLE I

Solubility of β-Lactoglobulins from Individual Cow's Milk in Water[a]

Type	No. samples	pH solution (avg)	Solubility (gm/1000 cc)	
			Range	Avg.
		25°		
A	7	5.35	0.60–0.69	0.61
B	9	5.25	2.90–3.50	3.1
AB	2	5.40	0.90–1.10	1.0
Pooled	1	5.38	1.49	1.49
		5°		
A	1	5.38	0.44	
B	1	5.40	1.60	
AB	1	5.37	0.65	

[a] Total protein: 12 gm/1000 cc.

forms of crystals without recrystallizing them together.

Values for the solubilities of β-lactoglobulins A, B, and AB from individual cow's milk as well as from pooled milk are recorded in Table I. The results show that type B is about 5 times more soluble than type A in water and that type AB and β-lactoglobulin from mixed milk have intermediate solubilities. Preliminary solubilities at 5° indicate that each of the β-lactoglobulins is about two thirds as soluble at 5° as at 25°. No previous quantitative results have been reported for the solubility of typed β-lactoglobulins in water. Palmer (1) found a value of 1.23 gm/1000 gm at 30°, while Grönwall (3) reported a value of 1.0 gm per liter at 25° for the solubility of β-lactoglobulin in water for untyped β-lactoglobulin.

The solubilities in water and sodium chloride solutions of a number of samples of β-lactoglobulin from individual cow's milk are illustrated in Figs. 4 and 5. Palmer (1) obtained a straight line with a slope of about 9 by plotting the logarithm of the solubility of β-lactoglobulin against the square root of the sodium chloride concentration. However, the extrapolation to zero salt gave a much lower solubility value than the determined value. Our solubility data also gives parallel straight lines with slopes of about 9 when the logarithms of the solubilities are plotted against the square root of the ionic strength with extrapolated values for zero salt of about one half of the determined values (Fig. 4). However, when the logarithm of the solubility is plotted against ionic strength, extrapolation to zero salt gives values which agree with the experimental results (Fig. 5), which was also found to be true by Grönwall (3). When the logarithm of the ratio of the solubility in salt (S_1) is divided by the solubility in water (S_0) for each sample, a single curve is obtained, as shown in Fig. 6, where the average values are plotted.

Deionized β-Lactoglobulin

Since the solubility data gives a much lower extrapolated value for the solubility in water than the determined value when it is plotted against the square root of ionic strength, it was thought that the samples might contain a small amount of salt, as suggested by Palmer (1) and made more probable by the findings of Tanford and Nozaki (16), i.e., that the isoionic point of β-lactoglobulin A is about 5.35 and B, 5.45, instead of the lower value of 5.20 found by Cannan *et al.* (17). Consequently, β-lactoglobulin preparations, dissolved in acetic acid, sodium chloride, or ammonium hydroxide, were freed from ions by passing down a mixed-bed, ion exchange column prepared according to Dintzis's procedure (18), which is similar to the procedure used by Tanford and Nozaki (16). The column was 2.0 cm in diameter and had a depth of 20 cm. A layer of Amberlite IR-120, H^+ form of 4 cm was always placed

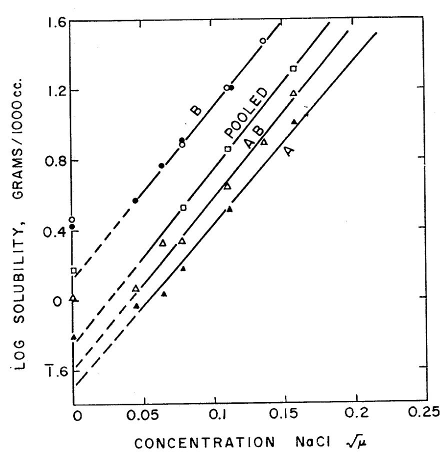

Fig. 4. Solubilities of β-lactoglobulins plotted as a function of the square root of the ionic strength.

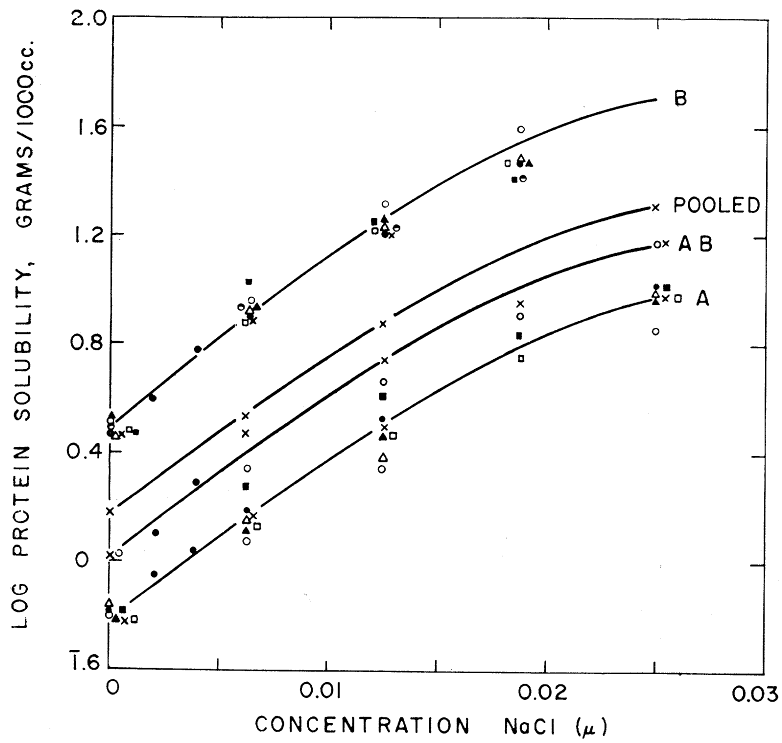

FIG. 5. Solubilities of β-lactoglobulins plotted as a function of ionic strength

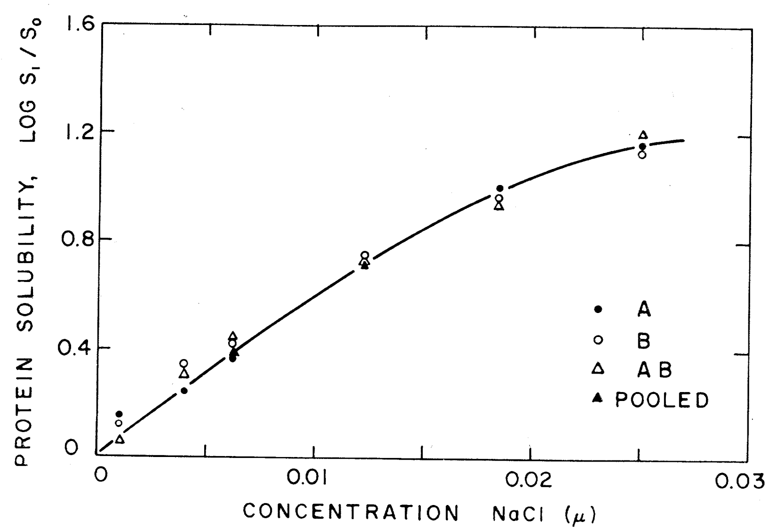

FIG. 6. Solubilities of β-lactoglobulins when the ratio of the solubility in salt is divided by the solubility in water; average values plotted as a function of ionic strength.

in the bottom of the column. β-Lactoglobulin solutions containing 1–2% protein were passed down the deionization column in the usual manner. The first values obtained on the deionized β-lactoglobulins were close to pH 5.20, irrespective of the type of β-lactoglobulin. However, by repetition and lowering the rate of flow, a small but significant difference in the isoionic values for β-lactoglobulins A and B were obtained, in qualita-